

VERIFICATION OF TRANSLATION

Re: JAPANESE PATENT APPLICATION NO. 1997-214604

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hereby declare that I am the translator of the  
document attached and certify that the following is  
true translation to the best of my knowledge and  
belief.

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[Document Name] Specification

[Title of the Invention] ISOFLAVONE-CONTAINING COMPOSITION

[Claims]

[Claim 1] An isoflavone-containing composition comprising  
5 a isoflavone and a strain of microorganism capable of  
metabolizing daidzein to equol.

[Claim 2] The isoflavone-containing composition according  
to Claim 1 wherein the strain of microorganism capable of  
metabolizing daidzein to equol is at least one member  
10 selected from the group consisting of *Bacteroides ovatus*,  
*Streptococcus intermedius* and *Streptococcus constellatus*.

[Claim 3] The isoflavone-containing composition according  
to Claim 1 or 2 which further contains at least one  
component that favors the maintenance and growth of the  
15 strain of microorganism capable of metabolizing daidzein  
to equol.

[Claim 4] The isoflavone-containing composition according  
to Claim 3 wherein the component that favors the  
maintenance and growth of the strain of microorganism  
20 capable of metabolizing daidzein to equol is at least one  
substance selected from the group consisting of lacto-  
oligosaccharide, soya oligosaccharide, lactulose, lactitol  
and fructo-oligosaccharide.

[Claim 5] An equol-containing composition comprising equol  
25 obtained by causing a strain of microorganism capable of

metabolizing daidzein to equol to act upon a isoflavone.

[Claim 6] The composition according to Claims 1-4 wherein  
the food form is selected from the group consisting of  
drinks, dairy products, fermented milk, bars, granules,  
5 powders, capsules and tablets.

[Detailed Description of the Invention]

[0001]

[Technical Field to Which the Invention Pertains]

The present invention relates to an isoflavone-  
10 containing composition and equal-containing composition,  
and more particularly to a novel composition either  
comprising blending an isoflavone such as diadzein,  
genistein, daidzin or genistin with a specific strain of  
microorganism or comprising equol, which composition is  
15 useful for the prevention and palliation of unidentified  
clinical symptoms in middle-aged to elderly women,  
inclusive of menopausal syndrome.

[0002]

[Prior Art]

20 The documented collaborative research of  
National Cancer Center of Japan and Helsinki University  
(Finland) attributes the low incidence of gender-specific  
carcinomas such as carcinoma of the prostate in men and  
carcinoma of the breast or ovary in women among the  
25 Japanese as compared with the European and American people

to the greater intake by the Japanese of soybean-derived foods and the consequent well-coordinated balance of hormones (H. Adlercreutz, et al., (1992) Lancet, 339, 1233; H. Adlercreutz, et al., (1992) Lancet, 342, 1209-  
5 1210).

[0003]

Recently, there has been a mounting interest in the fact that isoflavones have estrogen (female hormone)-like activity (A. Molteni, et al., (1995) J. Nutr., 125, 751S-756S), and it has been reported that these compounds  
10 are effective in osteoporosis which develops after the menopause when estrogen secretions have subsided or ceased (D. Agnusdei, et al., (1995) Bone and Mineral, 19 (Supple), S43-S48) as well as in menopausal syndrome (D. D. Baird,  
15 et al., (1995) J. Clin. Endocrinol, Metab., 80, 1685-1690; A. L. Murkies, et al., (1995) Maturitas., 21, 195-198).

[0004]

According to the result of a survey undertaken by Margaret Lock (M. Lock, et al., (1988) Maturitas., 10, 317-332), the incidence of climacteric symptoms among the  
20 Japanese women is extremely low as compared with the Canadian counterparts. Based on the report, H. Adlercreutz and coworkers conjecture that the Japanese women ingest large amounts of processed soybean foods such  
25 as tofu, miso, soy sauce, etc. and, hence, the plant

estrogens (isoflavones) contained in those foods are responsible for the low incidence of menopausal symptoms. The same authors further report that, comparing the urinary excretions (24-hour urine) which are known to reflect the amount of absorption of isoflavones actually ingested, the urinary excretions in the Japanese women are tens of times as high compared with the Western women (C. Herman, et al., (1995), J. Nutr., 125, 757S-770S).

[0005]

10           It is, thus, considered that the intake of isoflavones is effective for the palliation and prevention of postmenopausal osteoporosis and menopausal syndrome. Particularly, the postmenopausal life expectancy in women has reportedly increased to more than 30 years owing to the recent trend toward longevity and the alleviation and prevention of various diseases and symptoms which may develop after the menopause have important meanings in that they would lead to improvements in quality of life (QOL).

20   [0006]

          However, the above report, i.e. the survey report on the amount of intake of isoflavones and the urinary excretions of isoflavones in the middle-aged to elderly women in Japan reflects the results generated in a limited rural area and no substantive information is

available. Moreover, the correlation between the onset of menopausal syndrome and the amount of intake of isoflavones has not been squarely analyzed and revealed.  
[0007]

5 [Problem to be Solved by the Invention]

Therefore, the object of the present invention is to provide a novel composition which is effective for the prevention and palliation of the unidentified clinical symptoms in middle-aged to elderly women including the  
10 menopausal syndrome, for which no effective means of prophylaxis or relief has been available.  
[0008]

To accomplish the above object, the inventors of the present invention first conducted a diet or food menu  
15 survey, determination of urinary excretions of isoflavones, and a questionnaire survey about menopausal syndrome (unidentified clinical symptoms) in perimenopausal women in a broad geographical area including urban communities. According to the results of the above surveys conducted in  
20 116 women aged between 40 and 60 who belonged to Fukuoka Prefectural Dietitians Association, the average amounts of intake of isoflavones were 9 mg/day for daidzein and 13 mg/day for genistein. The average urinary excretions of isoflavones were 19.6  $\mu$ mol/day for daidzein, 10.0  $\mu$ mol/day  
25 for genistein, and 11.9  $\mu$ mol/day for equol (mean of

subjects in whom it was detected). Incidentally, although daidzein and genistein were detected in all the subjects, equol was detected only in 46 (51.6%) of the 95 subjects. [0009]

5                   Furthermore, women with paramenia and those within 5 years of the menopause being taken together as menopausal subjects, a questionnaire survey was conducted using 17 items which are in routine use in the diagnosis of menopausal syndrome and the simplified menopausal index  
10 (SMI) was calculated. With taking subjects with SMI values not less than 20 as a group of high climacteric symptoms and those with SMI values not greater than 19 as a group of low climacteric symptoms, the amount of intake of isoflavones and the urinary excretion of isoflavones  
15 were respectively compared between the groups. As a result, whereas no intergroup difference was found in the amount of intake of daidzein, the amount of intake of genistein tended to be lower in the group of high climacteric symptoms at  $p = 0.0643$ . With regard to the  
20 urinary excretions of isoflavones, no intergroup difference was found with respect to daidzein and genistein, but the excretions of equal were significantly low ( $p < 0.01$ ) in the group of high climacteric symptoms. [0010]

25                   The above results led the present inventors to

the finding that unidentified clinical symptoms in menopausal women are more closely related to the amount of intake of genistein and the urinary excretion of equal.

[0011]

5           In the past the relationship between the amounts of intake and urinary excretion of isoflavones and their physiological effect has been discussed without regard to specific kinds of isoflavones such as daidzein and genistein but the results of the survey conducted by the present inventors in the Japanese middle-aged and elderly women made it clear that not only the amounts of intake and urinary excretion of isoflavones in general but also the amount of intake of genistein and the urinary excretion of equol relate to the physiological effect of isoflavones, and that the rate of metabolic conversion of daidzein to equol is also closely related to unidentified clinical climacteric symptoms in menopausal women.

[0012]

20           In another study undertaken by the inventors in healthy adult volunteers (25~33 years of age), it was found that the urinary excretions of isoflavones (daidzein and genistein) after single ingestion of soy milk are increased in a dose-related fashion, whereas in subjects who showed no urinary excretion of equol, equol was not detected in the urine even when the amount of intake of



soy milk was increased two-fold, indicating the existence of individual difference in the metabolic pathway from daidzein to equol.

[0013]

5               Based on the above findings the present inventors conducted further research and, as a result, developed a novel composition which comprises equol which is not detected in processed soybean products nor is it taken into the body from foods in ordinary diets and a  
10 novel composition which comprises a strain of microorganism capable of metabolizing daidzein to equol and isoflavones such as daidzein in combination. The inventors then discovered that the intake of whichever of the above compositions is effective in the prevention and  
15 palliation of unidentified clinical symptoms in middle-aged and elderly women and have accordingly succeeded in providing a composition to meet the object.

[0014]

[Means for Solving the Problem]

20               The present invention provides an isoflavone-containing composition which is characterized by comprising an isoflavone and a strain of microorganism capable of metabolizing daidzein to equol.

[0015]

25               According to the present invention, there can be

provided the isoflavone-containing composition wherein the strain of microorganism capable of metabolizing daidzein to equol is at least one member selected from the group consisting of *Bacteroides ovatus*, *Streptococcus intermedius* and *Streptococcus constellatus*; the isoflavone-containing composition which further contains at least one component that favors the maintenance and growth of the strain of microorganism capable of metabolizing daidzein to equol; the isoflavone-containing composition wherein the component that favors the maintenance and growth of the strain of microorganism capable of metabolizing daidzein to equol is at least one substance selected from the group consisting of lacto-oligosaccharide, soya oligosaccharide, lactulose, lactitol and fructo-oligosaccharide; and the isoflavone-containing composition wherein the food form is selected from the group consisting of drinks, dairy products, fermented milk, bars, granules, powders, capsules and tablets.

[0016]

20           The present invention further provides an equol-containing composition which is characterized by comprising equol obtained by causing a strain of microorganism capable of metabolizing daidzein to equol to act upon a isoflavone, whose the food form is selected  
25   from the group consisting of drinks, dairy products,

fermented milk, bars, granules, powders, capsules and tablets.

[0017]

[Mode for Carrying out the Invention]

5           In the present invention, isoflavones derived from soybean, kudzu, their processed products and their fermentation products can be used as one ingredient (i.e. soya isoflavones) to construct the composition of the invention, but the isoflavones contained in plants such as  
10 red clove and alfalfa can also be used. The processed products include, for example, tofu, soy sauce, abura-age, soy milk and the like and the fermentation products include natto, miso, tempeh and the like.

[0018]

15           In the isoflavone-containing composition of the invention, a strain of microorganism having an ability (metabolic activity) to produce equol from daidzein is used as the other ingredient. The microorganism mentioned above includes those belonging to Bacteroides ovatus,  
20 Streptococcus intermedius, and Streptococcus constellatus. Particularly preferred among such microorganisms are Bacteroides E-23-15 (FERM P-16312), Streptococcus E-23-17 (FERM P-16313) and Streptococcus A6G-225 (FERM P-16314), all of which were isolated from human stools and deposited  
25 for accession by the present inventors.

[0019]

The above strain of microorganism may generally be the live microorganism as such. However, it is not limited thereto but includes its culture, a crude or  
5 purified product thereof, and their lyophilizates. Its proportion is not particularly restricted but can be judiciously selected according to the kind of microorganism, among other factors. For example, in the case of *Streptococcus intermedius* in fermented milk, the  
10 bacterial count is preferably controlled within the range of about  $10^8 \sim 10^9$  cells/mol. The bacterial count is determined by inoculating an agar medium with a diluted sample, incubating the inoculated medium anaerobically at 37°C and counting the colonies formed.

15 [0020]

The composition of the invention using the microorganisms capable of metabolizing daidzein to equol further preferably contains a nutrient component particularly suited to the maintenance and growth of the  
20 particular strain of microorganism. The nutrient component includes but is not limited to lacto-oligosaccharide, soya oligosaccharide, lactulose, lactitol, fructo-oligosaccharide, and the like. The formulating amount of such nutrients is not particularly restricted  
25 but generally is preferably selected from the range of

about 5~10 weight % based on the total composition of the invention.

[0021]

The composition of the invention is generally prepared by blending predetermined amounts of said ingredients and processing the mixture into a suitable form such as drinks, dairy products, fermented milk, bars, granules, powders, capsules, tablets and tablets. The blending ratio of isoflavones and specific strain of microorganism is not particularly critical. However, it is preferred in general to blend not less than 10 mg of isoflavone as genistein, 1~5 g of oligosaccharide and  $10^9 \sim 10^{10}$  cells (as viable cells) of microorganisms.

[0022]

Since the isoflavone-containing composition of the invention contains a strain of microorganism (primarily live cells) as mentioned above, the composition preferably should not be subjected to heating and/or pressurization in the course of processing into final products. Therefore, in processing the composition of the invention into such forms as bars, granules, powders, tablets, etc., it is preferable to add the microorganism as lyophilized cells as such or lyophilized cells coated with a suitable coating agent.

[0023]

Examples of other materials which may be blended to the composition of the invention include calcium, vitamin D, vitamin C, vitamin E and the like.

[0024]

5           Further, according to the present invention, an equol-containing composition comprising equol obtainable by causing a strain of microorganism capable of utilizing daidzein to produce equol to act upon a isoflavone, especially upon isoflavones derived from soya bean, kudzu  
10 and their processed food or fermented food is provided in the form of drinks, dairy products, fermented milk, bars, granules, powders, capsules, tablets and tablets.

[0025]

          The equol-containing composition of the  
15 invention is very safe because the active ingredient thereof is a native substance as mentioned above and also advantageous in that it can be obtained in high yield and at low production cost.

[0026]

20           Here, equol is prepared by fermentation of the isoflavones derived from soya isoflavone, kudzu, red clove and alfalfa, preferably food materials containing soya isoflavone utilizing the microorganisms. Examples of the food materials include tofu, soy milk, soya beans, soya  
25 bean embryo extracts and the like.

[0027]

More particularly, the preparation of equol from isoflavone comprises sterilizing the food materials in solution form, adding the predetermined strain of  
5 microorganism thereto, and incubating the mixture at 37°C either under anaerobic conditions or under aerobic stationary conditions for about 24-48 hours to let fermentation proceed [where necessary, a pH control agent, a reducing substance (e.g. yeast extract, vitamin K1) can  
10 be added].

[0029]

The form mentioned above includes but is not limited to drinks, milk products, fermented milk, bars, granules, powders, capsules, and tablets.

15 [0030]

The amount of equol in the resulting composition of the invention may preferably be about 10~50 mg based on 100 g of the total composition.

[0031]

20 The amount of intake of the composition of the invention is not particularly restricted but can be generally selected so that the urinary excretions of equol after ingestion of the composition will not be less than 5  $\mu$ M/day.

25 [0032]

[Example]

For a further detailed description of the invention, examples of preparation of foods of the invention, however, it is to be understood that the scope  
5 of the invention is by no means delimited by those specific examples.

[0033]

[Example 1] Preparation of a drink

The ingredients according to the following  
10 recipe were weighed and blended to provide the composition of the invention in the form of a beverage.

[0034]

water-soluble soybean protein	10 ml
Lacto-oligosaccharide (55% content)	10.0 g
15 Vitamins & minerals	q.s.
Flavor	q.s.
Water	q.s.
Total	150 ml

[0035]

20 [Example 2] Preparation of a fermented milk

The ingredients according to the following recipe were weighed and blended to provide the isoflavone-containing composition of the invention in the form of fermented milk.

25 [0036]



	Water-soluble soybean protein	2.2 g
	Lacto-oligosaccharide (55% content)	10.0 g
	Streptococcus A6G-225-fermented milk	100 ml
	Vitamins & minerals	q.s.
5	Flavor	q.s.
	Water	q.s.
	Total	50 ml

The Streptococcus·intermedius fermented milk was prepared by adding  $10^8$  cells of Streptococcus·intermedius (FERM P-16313) to 1 liter of milk and incubating the mixture at 37°C for 24 hours.

[0037]

[Example 3] Preparation of a fermented soy milk lyophilizate

15 Using 1 ml of a suspension of about  $10^7$  cells/ml of Streptococcus·intermedius (FERM P-16313), 100 g of soy milk was caused to undergo lactic acid fermentation at 37°C for 24 hours to provide equol. This product was lyophilized. The equol content of this freeze-dried powder was 0.1~0.3 weight %.

[0038]

The above powder and other ingredients according to the following recipe were weighed and blended to provide the composition of the invention in the form of a fermented soy milk lyophilizate.

[0039]

	Fermented soy milk lyophilizate	2.2 g
	Excipient	q.s.
	Vitamins & minerals	q.s.
5	Flavor	q.s.
	Total	20 g

As the excipient, 17 g of cornstarch was used.

[0040]

[Example 4] Preparation of powders

10           The ingredients according to the following  
recipe were weighed and blended to provide the composition  
of the invention in powdery form.

[0041]

	Crude soya isoflavone powder	4.1 g
15	Lacto-oligosaccharide (55% content)	10.0 g
	Streptococcus E-23-17 lyophilizate	1.0 g
	Vitamins & minerals	q.s.
	Flavor	q.s.
	Total	20 g

20           The Streptococcus·intermedius lyophilizate was  
prepared by growing Streptococcus·intermedius fermented  
milk of Example 2 or Streptococcus·intermedius (FERM P-  
16313) in a suitable liquid growth medium (GAM broth)  
(37°C, 24~48 hours) and then lyophilizing the resulting  
25 culture. The bacterial cell content of this freeze-dried

powder was  $10^9 \sim 10^{10}$  cells/g.

[0042]

[Example 5] Preparation of granules

The ingredients according to the following  
5 recipe were weighed and blended to provide the composition  
of the invention in granular form.

[0043]

	Crude soya isoflavone powder	4.1 g
	Lacto-oligosaccharide (55% content)	10.0 g
10	Streptococcus E-23-17 lyophilizate	1.0 g
	Sorbitol	q.s.
	Vitamins & minerals	q.s.
	Flavor	q.s.
	Total	20 g

15 As the Streptococcus intermedius lyophilizate,  
the same freeze-dried powder as in Example 4 was used.

[Document Name] Abstract

[Abstract]

[Object] This invention provides a novel composition which is effective in the prevention and palliation of  
5 unidentified clinical symptoms inclusive of menopausal syndrome in middle-aged to elderly women for which no effective means of prophylaxis or relief has heretofore been available.

[Method for Achieving the Object] An isoflavone-containing  
10 composition comprising isoflavone in combination with a strain of microorganism capable of metabolizing daidzein to equol or an equol-containing composition comprising equol.

[Selected Figure] None